Conservative pattern of interaction of bat and human IgG antibodies with FcRn

Nia Toshkova, Violeta Zhelyazkova, Sune Justesen, Jordan Dimitrov

Conservative pattern of interaction of bat and human IgG antibodies with FcRn

Nia Toshkova[1,2], Violeta Zhelyazkova[1,3], Sune Justesen[4], & Jordan D. Dimitrov[3]

[1]National Museum of Natural History, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel Blvd., 1000 Sofia, Bulgaria

[2]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel Blvd., 1000 Sofia, Bulgaria

[3]Centre de Recherche des Cordeliers, INSERM, CNRS, Sorbonne Université, Université de Paris, 75006 Paris, France

[4]Immunitrack Aps, Lersoe Park Alle 42, 2100, Copenhagen East, Denmark

Correspondence to:

Jordan D. Dimitrov
INSERM UMRS 1138,
Centre de Recherche des Cordeliers,
75006 Paris, France.
Tel: +33 1 44 27 82 06
E-mail: jordan.dimitrov@sorbonne-universite.fr

or

Nia Toshkova
National Museum of Natural History,
Bulgarian Academy of Sciences,
1000 Sofia, Bulgaria
Tel: +359 89 40 69 084
E-mail: niyatoshkova@gmail.com

Abstract

Recently, numerous studies report bats as reservoirs of emerging pathogens with little to no signs of infections. This is thought to be connected to the unique immune system of bats, which remains poorly characterized. Despite the physiological importance of the Neonatal Fc receptor (FcRn) in the homeostasis of IgG antibodies, it is unclear how its functional activity is evolutionary conservative among mammals, and so is the case for bats. Using surface plasmon resonance-based technology, we tested the interactions of IgG antibodies isolated from three bat species with recombinant human and mouse FcRn. Our data show that IgG from the studied bat species binds to both human and mouse FcRn, albeit with distinct affinities. Importantly, the binding pattern of bat IgG is similar to human IgG. This confirms the conservative nature of IgG-FcRn interaction and highlights the importance of FcRn IgG salvaging system in bats.

Keywords:

Immunoglobulins, IgG, neonatal Fc Receptor, Bat immunity, surface plasmon resonance

Introduction

Chiroptera (bats) is the second most diverse order of mammals. Species from this order have been recently identified as important model organisms due to the unique properties of their immune system. Various idiosyncratic properties of immune system of bats have been reported in the literature (Banerjee et al., 2020; Irving et al., 2021; Letko et al., 2020). They include a particular organization and regulation of interferon (IFN) pathways and IFN-signalling (Banerjee et al., 2020; Irving et al., 2021; Letko et al., 2020); dampened pro-inflammatory responses mediated by inflammasome (Ahn et al., 2019; Goh et al., 2020); impaired responses to DNA due to absence of PYHIN genes and a mutation in the adaptor protein STING (Ahn et al., 2016; Xie et al., 2018); higher levels of inhibitory receptors on NK cells; more diverse set of MHC I genes (Pavlovich et al., 2018), and different ratios of immune cells in circulation as compared to other mammals (Martinez Gomez et al., 2016; Periasamy et al., 2019). Generally immune response in bats against viruses have a tendency to be skewed towards tolerance rather than inducing strong inflammatory reactions, as it is observed in rodents and humans (Irving et al., 2021). These balanced innate inflammatory responses were proposed to appear as result of evolution of powered flight in bats. Notably, there is a long co-evolution of bats and pathogens. Moreover, bats are recognized as reservoirs of emerging pathogens, a fact that can be explained by their ostensible tolerance to viral infections and other particularities of their immune system (Banerjee et al., 2020; Irving et al., 2021; Letko et al., 2020). Despite this, the adaptive immune response of bats, which is necessary for building long-term immunity and resistance to viral infections, remains poorly characterized. Some studies have reported that antibody responses in bats are not stable and the plasma antibody titers are rapidly waning (Hatten et al., 1968; Schuh et al., 2017). In addition, the presence of

neutralizing antibodies might not be essential for control of certain viral infections (Schuh et al., 2019; Schuh et al., 2017). On the other side it was found that bats have a higher diversity of gene segments encoding variable domain of their immunoglobulins as compared to the gene diversity in human (Bratsch et al., 2011). A better understanding of the molecular and cellular interactions that compose the immune system of bats would significantly advance knowledge on the diversity and evolution of pathogen defence mechanisms in mammals. So far, the major isotypes of antibodies (Abs), immunoglobulin IgM, IgG, IgA, IgE, and IgD have been detected in several bat species (Baker et al., 2010; Butler et al., 2011; Chakravarty and Sarkar, 1994). IgG is the most abundant class of Ab in the serum of mammals. IgG antibodies are product of antigen-specific immune responses and they are crucial for humoral immune defence against pathogens (Lu et al., 2018). IgG is the only class of Ab that is known to be transferred from the mother to the offspring during the foetal development. This transfer occurs due to the specific interaction of IgG with the neonatal Fc receptor (FcRn) (Roopenian and Akilesh, 2007). Besides transferring humoral protection to neonates, FcRn has other important biological functions. Like so, it is involved in rescuing IgG (and albumin) from degradation in the lysosomes after pinocytosis by endothelial cells. This function of FcRn explains the considerably prolonged half-life of IgG and albumin (21 days in humans), as compared to other immunoglobulin isotypes or other plasma proteins (Roopenian and Akilesh, 2007; Ward and Ober, 2009). In addition to its functions in transplacental transfer and extension of circulatory half-life of IgG, FcRn plays a role in the phagocytosis of antigen-IgG immune-complexes and presentation of antigens by professional antigen-presenting cells (Baker et al., 2013; Stapleton et al., 2015; Vidarsson et al., 2006).

There are reports that FcRn is involved in the recycling and extension of the half-life of IgG in many mammals (Catunda Lemos et al., 2012; Ward and Ober, 2009). However, little is known

about this receptor in bats. It is unclear how evolutionary conservative and consistent the functions of FcRn are among mammals. The goal of the present study was to provide qualitative data that can contribute to characterization of the axis IgG-FcRn in bats. We isolated IgG from three European bat species (*Myotis myotis*, *Myotis capaccinii,* and *Nyctalus noctula*) and tested their interactions with recombinant human and mouse FcRn, using surface plasmon resonance-based technology. Our data revealed that IgG molecules from all studied bat species bind to human and mouse FcRn, albeit with distinct affinities and kinetics profiles. Importantly, the study revealed that the interaction of bat IgG with human FcRn was similar to that between human IgG and human FcRn. These data could be explained by the higher level of sequence homology between human and bat FcRn. To our knowledge, this is the first *in vitro* validation of the interaction of bat IgG with FcRn. Our study confirms the conservative nature of IgG antibodies in terms of their capacity to interact with FcRn. These data might contribute to a better understanding of humoral immune response in bats and the evolution of the FcRn receptor system in mammals.

Materials and methods

Sample collection

We conducted surveys in May and June 2022 in two Bulgarian caves: Ivanova voda (N41.894, E24.880) and Devetashata peshtera (N43.233, E24.885). From the first roost, we collected samples from greater moused-eared bats (*Myotis myotis*) (n=31) and long-fingered bats (*Myotis capaccinii*) (n=20). From the second roost, we collected samples from common noctule bats (*Nyctalus noctula*) (n=18). Bats were caught at the cave entrance at sunset using a harp trap and mist nets, then they were kept in individual cotton bags until processing. Their sex, size, weight, and approximate age were noted. Blood samples were taken from the uropatagium vein by puncturing it with a sterile disposable insulin needle and collecting the few drops of leaked blood using a pipette with sterile disposable tips. Blood sampling of this kind should not affect the survival rates of bats (Wimsatt et al., 2005). Serum was separated from coagulated blood by centrifugation and frozen immediately at -20°C. To obtain enough material for the experiments, pools of sera from up to 10 individuals were prepared. We followed all ethical requirements for working with bats and we used appropriate personal protective equipment. The research was carried out under two permits by the Bulgarian Biodiversity Act (No 830/19.09.2020 and No 927/04.04.2022). Additionally, blood samples were obtained from four anonymized healthy human donors under the ethical convention between INSERM with Etablissement Français du Sang (15/EFS/012). Prior to IgG purification, bat samples were processed in a L2 lab.

IgG purification

IgG from humans and bats was purified using MelonTM Gel IgG Spin Purification Kit (Thermo Fischer Scientific, Waltham, MA) according to the protocol recommended by the manufacturer,

with the exception that centrifugation was performed for 30 instead of 10 sec. The concentration of purified samples was estimated by a Nanodrop spectrophotometer (Thermo Fischer Scientific). To confirm the presence of IgG, a small fraction of the samples was separated on a Nu-PAGE 4-12% bis-tris gel electrophoresis, with or without a reducing agent (β-mercaptoethanol)., Proteins from the gel were then transferred to a nitrocellulose membrane using an electrotransfer machine (i-Blot, Invitrogen, Thermo Fischer Scientific) and blocked overnight with PBS containing 0.1 % Tween 20. The membranes were then probed with biotinylated Protein G (Thermo Fischer Scientific). Following extensive washing of the membranes with PBS containing 0.1% Tween 20, the bound protein G was detected by streptavidin conjugated with alkaline phosphatase (Southern Biotech, Birmingham, AL) and BCIP/NBT substrate system (Sigma-Aldrich, St. Louis, MI).

Surface plasmon resonance analyses

We analysed the binding kinetics of human and bat IgGs with hFcRn and mFcRn using surface plasmon resonance-based technology (Biacore 2000 system: Biacore Cytiva, Uppsala, Sweden). We immobilized with uniform orientation recombinant human and mouse FcRn receptors on a sensor chip, via binding of site-specifically biotinylated receptors (Immunitrack; see (Andersen et al., 2008a; Andersen et al., 2008b)) to pre-immobilized streptavidin. To evaluate the kinetics of binding of IgG from different bat species, we measured the real-time interaction binding profiles obtained after injection of increasing concentrations of IgG, following the experimental procedure as described in (Rossini et al., 2020) with some modifications. In brief, hFcRn and mFcRn were diluted to 10 µg/ml in running buffer (100 mM Tris pH 6.0, 100 mM NaCl, and 0.1 % Tween 20) and immobilized on SA sensor chip (Biacore Cytiva) with density of ca. 2 ng/mm^2. We performed these analyses at pH 6.0, a pH value of early endosomes, where the interaction of FcRn with IgG molecules typically occurs. Human and bat IgG was diluted in the running buffer in concentration

range 50 – 0.097 nM (2 × dilution step) and injected over the sensor surface with a flow rate of 30 µl/min. The association and dissociation phases were followed for 4 and 5 min, respectively. After each injection, the sensor surface was regenerated by injection for 30 sec of solution of the running buffer with pH 7.8. All kinetic measurements were performed at 22 °C. The binding kinetics of bat and human IgG to mFcRn and hFcRn was evaluated by applying global analyses using Langmuir binding model BIAevaluation version 4.1.1 Software (Biacore). For measuring binding of human and bat IgGs to FcRn at physiological pH, we used the same running and sample dilution buffer, but with pH 7.4. Human and bat IgGs were diluted to a single concentration of 50 nM and injected over the sensor chip with pre-coated mFcRn and hFcRn. The association and dissociation phases were recorded for 4 and 5 min, respectively.

Protein sequence homology analyses

To understand better the functional similarity between bat and human IgG in terms of binding to FcRn, we performed protein sequence homology analyses using the UniProt Align tool, https://www.uniprot.org/ (UniProt, 2021).

Results and discussion

Bat IgG purification

Here we report a successful application of Melon™ IgG purification system (Thermo Fischer) for the isolation of total IgG from sera of three European bat species: *Myotis capaccinii, Myotis myotis,* and *Nyctalus noctula*. The yield and purity of IgG were similar for the different bat species and for humans. This is an important observation demonstrating the feasibility of Melon™ system for purification of IgG from bats. Additionally, we successfully detected isolated bat IgG using Protein G, the strength of the detection signal being like this of human IgG. As Protein G recognises IgGs from a variety of mammal species, this system is very useful for experiments aiming interspecies comparison.

Bat IgG – FcRn interaction

To assess the interaction of bat IgG with human and mouse FcRn receptors, we applied surface plasmon resonance-based biosensor technology. To our knowledge, this is the first study where the binding to FcRn was investigated for bat IgGs. The interaction analyses clearly indicated that both human and mouse FcRn could bind with substantial affinities to IgG isolated from *Myotis capaccinii, Myotis myotis,* and *Nyctalus noctula* (Fig. 1A). This result provides important evidence about the conservative nature of IgG – FcRn interaction in mammals and suggests that bat IgG can also be protected from catabolism by FcRn and thereby characterized with a long half-life as is the case in other mammals.

The binding profiles from biosensor analyses indicated that mouse FcRn (mFcRn) binds bat and human IgGs with a higher intensity as compared to human FcRn (hFcRn) (Fig. 1A). Similar

tendency was observed while studying the interactions of human IgG. Next, by applying global kinetic analyses we evaluated the kinetic parameters and the binding affinities (K_D values) of the interactions of bat and human IgG with mFcRn and hFcRn (Fig. 1B). These analyses demonstrated that IgG from the three studied bat species bind to hFcRn with high affinities (K_D ranging between ca. 10 – 18 nM) and with very similar kinetics. These values were slightly lower than the one detected for binding of human IgG to hFcRn (K_D of 4 nM) but they are well within the broad range of values of affinity (K_D from μM to sub nM) reported in the literature (Grevys et al., 2018; Piche-Nicholas et al., 2018; Rossini et al., 2020; Schoch et al., 2015; Sun et al., 2020; Wang et al., 2011). The binding of IgG isolated from different bat species to mFcRn was characterized by a higher affinity than the binding to hFcRn. Thus, the obtained values of K_D were in the range of 1.1 to 2.8 nM (Fig. 1B). The binding affinity of human IgG was also considerably higher when binding to mFcRn as compared to hFcRn. The kinetic analyses revealed that the observed differences in the affinity between FcRn receptors from mouse and human were due to differences in the value of the dissociation rate constants. Indeed, the IgG molecules from different bat species and human IgG bind both hFcRn and mFcRn with almost identical values of k_a. In contrast, the dissociation rate constant of IgGs from mFcRn was substantially lower (at least an order of difference, Fig. 1). The observation that at acidic pH mFcRn binds with a higher affinity human IgG than hFcRn confirms previous findings from the literature (Abdiche et al., 2015). Interestingly, our work revealed that this effect is also present in the case of IgG antibodies from different species of bats. The increased affinity of mFcRn for IgG from distinct species could be explained by specific structural and physicochemical features of the receptor. It also strongly implied that bat IgGs prove to be functionally conservative in respect to binding to FcRn. These data suggest that model systems with hFcRn may be more relevant for studies of bat IgG as compared to cells of mouse

origin. In this respect, it was found that the transgenic mouse model expressing hFcRn is relevant model for studying pharmacokinetics of human IgG (Avery et al., 2016). These transgenic mice might be a relevant tool for *in vivo* studies with bat IgG antibodies.

Efficient salvaging of IgG by FcRn requires that the receptor binds IgG at acidic pH (pH <6.5) and releases its cargo at physiological pH (pH 7.4) (Roopenian and Akilesh, 2007). Accordingly, previous works demonstrated that IgG antibodies that bind to FcRn with high-affinities both at acidic and physiological pH are characterized by a shortened half-life in circulation due to their retention to FcRn and subsequent endosomal degradation (Schoch et al., 2015). To elucidate whether the binding of bat IgGs to FcRn follows the typical pH dependency, we compared the interactions at pH 6.0 and pH 7.4 (Fig. 1C) using real-time binding analyses. The obtained data indicated that human and bat IgG almost completely lost their capacity to bind hFcRn at physiological pH. In contrast, substantially higher amounts of IgG (as deduced by higher remaining resonance units at the end of the association phase of interactions) were retained by mFcRn at physiological pH, especially in the case of IgG from *M. capaccinii* and *N. noctula* (Fig. 1C). This result implies that bat IgG can be efficiently recycled by hFcRn, similar to human IgG, but not by mFcRn.

Protein sequence homology analyses

To understand the origin of the observed differences in the interaction of bat IgG with hFcRn and mFcRn, we performed sequence homology analyses of FcRn receptors from different species. We performed the homology analyses using a sequence of FcRn from only one bat species (*M. myotis*), as sequences of FcRn are not available for the other two bat species used in the present study. These analyses demonstrated that FcRn receptors of bats (*M. myotis*), mice (*M. musculus*) and

humans are characterized by relatively high sequence homology (Figure 2). The highest sequence homology i.e., 70.62 %, was observed between human and bat FcRn, while human and mouse FcRn are characterized by a sequence homology of 66.39 %. On the other hand, the sequence homology between the bat and the mouse FcRn was the lowest, 63.35 %. A higher level of sequence homology between bat and human FcRn can explain the observed similarity in the binding behaviour to FcRn receptors IgG antibodies from these species. These data also suggest that the bat IgG can be recycled and characterized with long half-life similarly as human IgG. Notably, a comparative analysis of the expression patterns of immune-related genes in different immune cells isolated from human, mouse and cave nectar bat (*Eonycteris spelaea*), revealed a higher phylogenetic similarity between human and bat than between human and mice immune cells (Gamage et al., 2020). Our study provides an additional example for a functional similarity in human and bats of an essential immune-related molecular system.

Concluding remarks

The conservative nature of the interaction of IgG with FcRn in different species of mammals underlines its importance in the evolution of the immune system. The versatile biological functions of FcRn, ranging from transfer of protective IgG antibodies from mother to offspring, extension of the circulatory half-life of IgG and albumin, presentation of antigens, to the establishment of immune tolerance, makes this receptor an essential and indispensable component of the immune system. Our study provides evidence that FcRn-IgG interaction is conservative in the evolution. The data also suggest that this interaction may play an important role in the immune system of bats. Further studies should be dedicated on cloning and expression of recombinant bat FcRn receptors and functional characterization of their interaction with bat IgG. In addition, we show

that the usage of human cell lines or transgenic mice with human FcRn may be a more relevant model for the study of bat IgG antibodies as compared to cells with murine origin. Lastly, bats are mammals with unusually high resistance to viral infections and are considered reservoirs of various pathogens that can seriously affect humans. Thus, advancing our knowledge of bat humoral immune system might be valuable for protecting public health in the future.

Acknowledgments

We would like to acknowledge the volunteers that help us in the field: Boris Petrov, Petar Velkov, Katrin Dimitrova, and Kristin Meshinska. We are also thankful to Lubka Roumenina for her support regarding the protein sequence homology analyses.

Funding

Funding for this study (especially for the field work) was provided by the Bulgarian National Science Fund (project КП-06-Н51/9 "Caves as a reservoir for novel and reoccurring zoonoses - ecological monitoring and metagenomic analysis in real-time"). NT was funded by a Ph.D. Fellowship from Karoll Knowledge Foundation and a stipend from the French Institute, Bulgaria. VZ was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Young scientists and postdoctoral students" (DCM 577/ 17.08.2018) and a stipend from the French Institute, Bulgaria.

Conflicts of interest

The authors have no conflicts of interest to declare.

Data availability

Source files used for evaluation of the kinetic data are available upon request.

References

Abdiche, Y.N., Yeung, Y.A., Chaparro-Riggers, J., Barman, I., Strop, P., Chin, S.M., Pham, A., Bolton, G., McDonough, D., Lindquist, K., Pons, J., Rajpal, A., 2015. The neonatal Fc receptor (FcRn) binds independently to both sites of the IgG homodimer with identical affinity. MAbs 7, 331-343.

Ahn, M., Anderson, D.E., Zhang, Q., Tan, C.W., Lim, B.L., Luko, K., Wen, M., Chia, W.N., Mani, S., Wang, L.C., Ng, J.H.J., Sobota, R.M., Dutertre, C.A., Ginhoux, F., Shi, Z.L., Irving, A.T., Wang, L.F., 2019. Dampened NLRP3-mediated inflammation in bats and implications for a special viral reservoir host. Nat Microbiol 4, 789-799.

Ahn, M., Cui, J., Irving, A.T., Wang, L.F., 2016. Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. Sci Rep 6, 21722.

Andersen, J.T., Justesen, S., Berntzen, G., Michaelsen, T.E., Lauvrak, V., Fleckenstein, B., Buus, S., Sandlie, I., 2008a. A strategy for bacterial production of a soluble functional human neonatal Fc receptor. J Immunol Methods 331, 39-49.

Andersen, J.T., Justesen, S., Fleckenstein, B., Michaelsen, T.E., Berntzen, G., Kenanova, V.E., Daba, M.B., Lauvrak, V., Buus, S., Sandlie, I., 2008b. Ligand binding and antigenic properties of a human neonatal Fc receptor with mutation of two unpaired cysteine residues. FEBS J 275, 4097-4110.

Avery, L.B., Wang, M., Kavosi, M.S., Joyce, A., Kurz, J.C., Fan, Y.Y., Dowty, M.E., Zhang, M., Zhang, Y., Cheng, A., Hua, F., Jones, H.M., Neubert, H., Polzer, R.J., O'Hara, D.M., 2016. Utility of a human FcRn transgenic mouse model in drug discovery for early assessment and prediction of human pharmacokinetics of monoclonal antibodies. MAbs 8, 1064-1078.

Baker, K., Rath, T., Flak, M.B., Arthur, J.C., Chen, Z., Glickman, J.N., Zlobec, I., Karamitopoulou, E., Stachler, M.D., Odze, R.D., Lencer, W.I., Jobin, C., Blumberg, R.S., 2013. Neonatal Fc

receptor expression in dendritic cells mediates protective immunity against colorectal cancer. Immunity 39, 1095-1107.

Baker, M.L., Tachedjian, M., Wang, L.F., 2010. Immunoglobulin heavy chain diversity in Pteropid bats: evidence for a diverse and highly specific antigen binding repertoire. Immunogenetics 62, 173-184.

Banerjee, A., Baker, M.L., Kulcsar, K., Misra, V., Plowright, R., Mossman, K., 2020. Novel Insights Into Immune Systems of Bats. Front Immunol 11, 26.

Bratsch, S., Wertz, N., Chaloner, K., Kunz, T.H., Butler, J.E., 2011. The little brown bat, M. lucifugus, displays a highly diverse V H, D H and J H repertoire but little evidence of somatic hypermutation. Dev Comp Immunol 35, 421-430.

Butler, J.E., Wertz, N., Zhao, Y., Zhang, S., Bao, Y., Bratsch, S., Kunz, T.H., Whitaker, J.O., Jr., Schountz, T., 2011. The two suborders of chiropterans have the canonical heavy-chain immunoglobulin (Ig) gene repertoire of eutherian mammals. Dev Comp Immunol 35, 273-284.

Catunda Lemos, A.P., Cervenak, J., Bender, B., Hoffmann, O.I., Baranyi, M., Kerekes, A., Farkas, A., Bosze, Z., Hiripi, L., Kacskovics, I., 2012. Characterization of the rabbit neonatal Fc receptor (FcRn) and analyzing the immunophenotype of the transgenic rabbits that overexpresses FcRn. PLoS One 7, e28869.

Chakravarty, A.K., Sarkar, S.K., 1994. Immunofluorescence analysis of immunoglobulin bearing lymphocytes in the Indian fruit bat: Pteropus giganteus. Lymphology 27, 97-104.

Gamage, A.M., Zhu, F., Ahn, M., Foo, R.J.H., Hey, Y.Y., Low, D.H.W., Mendenhall, I.H., Dutertre, C.A., Wang, L.F., 2020. Immunophenotyping monocytes, macrophages and granulocytes in the Pteropodid bat Eonycteris spelaea. Sci Rep 10, 309.

Goh, G., Ahn, M., Zhu, F., Lee, L.B., Luo, D., Irving, A.T., Wang, L.F., 2020. Complementary regulation of caspase-1 and IL-1beta reveals additional mechanisms of dampened inflammation in bats. Proc Natl Acad Sci U S A 117, 28939-28949.

Grevys, A., Nilsen, J., Sand, K.M.K., Daba, M.B., Oynebraten, I., Bern, M., McAdam, M.B., Foss, S., Schlothauer, T., Michaelsen, T.E., Christianson, G.J., Roopenian, D.C., Blumberg, R.S., Sandlie, I., Andersen, J.T., 2018. A human endothelial cell-based recycling assay for screening of FcRn targeted molecules. Nat Commun 9, 621.

Hatten, B.A., Allen, R., Sulkin, S.E., 1968. Immune response in chiroptera to bacteriophage phi-X174. J Immunol 101, 141-150.

Irving, A.T., Ahn, M., Goh, G., Anderson, D.E., Wang, L.F., 2021. Lessons from the host defences of bats, a unique viral reservoir. Nature 589, 363-370.

Letko, M., Seifert, S.N., Olival, K.J., Plowright, R.K., Munster, V.J., 2020. Bat-borne virus diversity, spillover and emergence. Nat Rev Microbiol 18, 461-471.

Lu, L.L., Suscovich, T.J., Fortune, S.M., Alter, G., 2018. Beyond binding: antibody effector functions in infectious diseases. Nature reviews. Immunology 18, 46-61.

Martinez Gomez, J.M., Periasamy, P., Dutertre, C.A., Irving, A.T., Ng, J.H., Crameri, G., Baker, M.L., Ginhoux, F., Wang, L.F., Alonso, S., 2016. Phenotypic and functional characterization of the major lymphocyte populations in the fruit-eating bat Pteropus alecto. Sci Rep 6, 37796.

Pavlovich, S.S., Lovett, S.P., Koroleva, G., Guito, J.C., Arnold, C.E., Nagle, E.R., Kulcsar, K., Lee, A., Thibaud-Nissen, F., Hume, A.J., Muhlberger, E., Uebelhoer, L.S., Towner, J.S., Rabadan, R., Sanchez-Lockhart, M., Kepler, T.B., Palacios, G., 2018. The Egyptian Rousette Genome Reveals Unexpected Features of Bat Antiviral Immunity. Cell 173, 1098-1110 e1018.

Periasamy, P., Hutchinson, P.E., Chen, J., Bonne, I., Shahul Hameed, S.S., Selvam, P., Hey, Y.Y., Fink, K., Irving, A.T., Dutertre, C.A., Baker, M., Crameri, G., Wang, L.F., Alonso, S., 2019. Studies on B Cells in the Fruit-Eating Black Flying Fox (Pteropus alecto). Front Immunol 10, 489.

Piche-Nicholas, N.M., Avery, L.B., King, A.C., Kavosi, M., Wang, M., O'Hara, D.M., Tchistiakova, L., Katragadda, M., 2018. Changes in complementarity-determining regions significantly alter IgG binding to the neonatal Fc receptor (FcRn) and pharmacokinetics. MAbs 10, 81-94.

Roopenian, D.C., Akilesh, S., 2007. FcRn: the neonatal Fc receptor comes of age. Nature reviews. Immunology 7, 715-725.

Rossini, S., Noe, R., Daventure, V., Lecerf, M., Justesen, S., Dimitrov, J.D., 2020. V Region of IgG Controls the Molecular Properties of the Binding Site for Neonatal Fc Receptor. J Immunol 205, 2850-2860.

Schoch, A., Kettenberger, H., Mundigl, O., Winter, G., Engert, J., Heinrich, J., Emrich, T., 2015. Charge-mediated influence of the antibody variable domain on FcRn-dependent pharmacokinetics. Proc Natl Acad Sci U S A 112, 5997-6002.

Schuh, A.J., Amman, B.R., Sealy, T.K., Kainulainen, M.H., Chakrabarti, A.K., Guerrero, L.W., Nichol, S.T., Albarino, C.G., Towner, J.S., 2019. Antibody-Mediated Virus Neutralization Is Not a Universal Mechanism of Marburg, Ebola, or Sosuga Virus Clearance in Egyptian Rousette Bats. J Infect Dis 219, 1716-1721.

Schuh, A.J., Amman, B.R., Sealy, T.K., Spengler, J.R., Nichol, S.T., Towner, J.S., 2017. Egyptian rousette bats maintain long-term protective immunity against Marburg virus infection despite diminished antibody levels. Sci Rep 7, 8763.

Stapleton, N.M., Einarsdottir, H.K., Stemerding, A.M., Vidarsson, G., 2015. The multiple facets of FcRn in immunity. Immunol Rev 268, 253-268.

Sun, Y., Estevez, A., Schlothauer, T., Wecksler, A.T., 2020. Antigen physiochemical properties allosterically effect the IgG Fc-region and Fc neonatal receptor affinity. MAbs 12, 1802135.

UniProt, C., 2021. UniProt: the universal protein knowledgebase in 2021. Nucleic Acids Res 49, D480-D489.

Vidarsson, G., Stemerding, A.M., Stapleton, N.M., Spliethoff, S.E., Janssen, H., Rebers, F.E., de Haas, M., van de Winkel, J.G., 2006. FcRn: an IgG receptor on phagocytes with a novel role in phagocytosis. Blood 108, 3573-3579.

Wang, W., Lu, P., Fang, Y., Hamuro, L., Pittman, T., Carr, B., Hochman, J., Prueksaritanont, T., 2011. Monoclonal antibodies with identical Fc sequences can bind to FcRn differentially with pharmacokinetic consequences. Drug Metab Dispos 39, 1469-1477.

Ward, E.S., Ober, R.J., 2009. Chapter 4: Multitasking by exploitation of intracellular transport functions the many faces of FcRn. Adv Immunol 103, 77-115.

Wimsatt, J., O'Shea, T.J., Ellison, L.E., Pearce, R.D., Price, V.R., 2005. Anesthesia and blood sampling of wild big brown bats (eptesicus fuscus) with an assessment of impacts on survival. J Wildl Dis 41, 87-95.

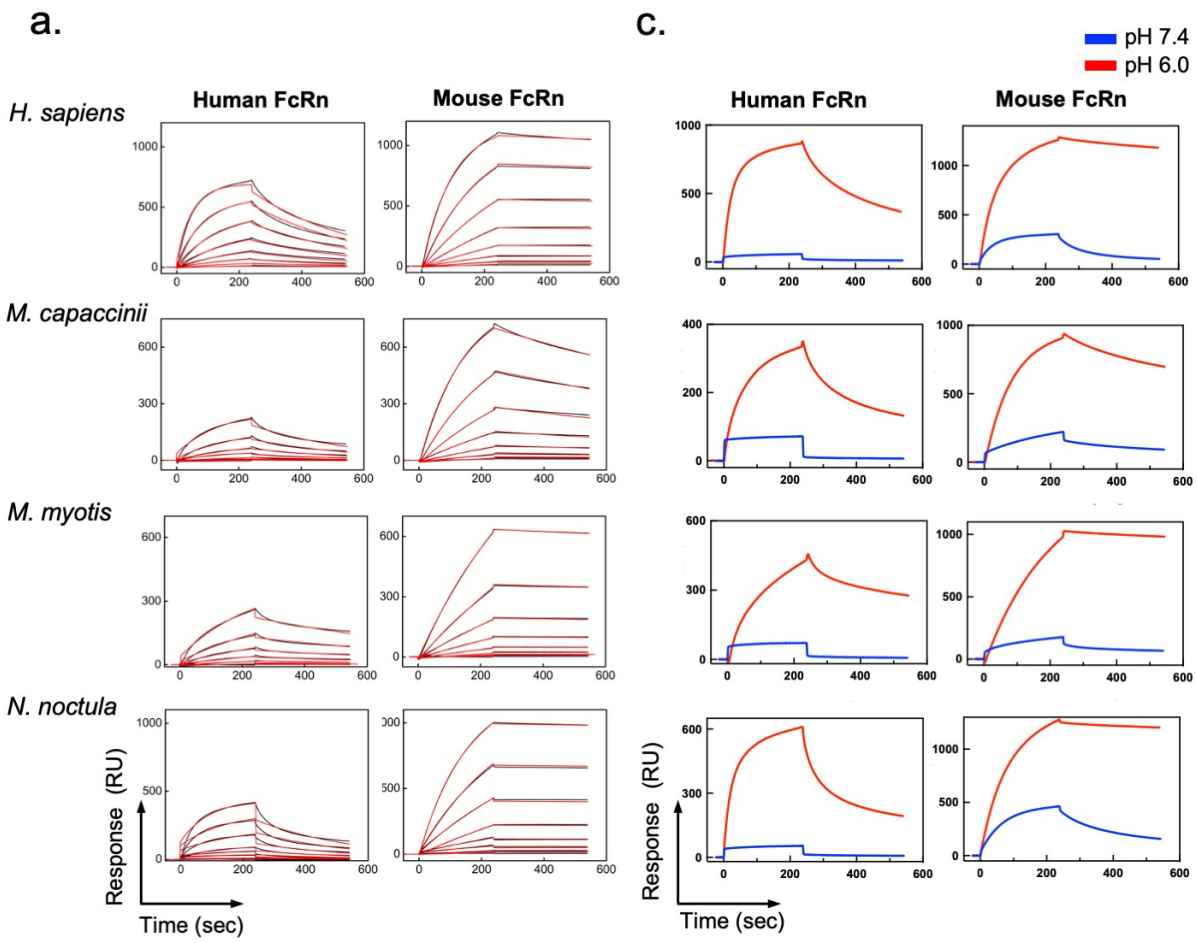
Xie, J., Li, Y., Shen, X., Goh, G., Zhu, Y., Cui, J., Wang, L.F., Shi, Z.L., Zhou, P., 2018. Dampened STING-Dependent Interferon Activation in Bats. Cell Host Microbe 23, 297-301 e294.

Legends to figures

Figure 1. Kinetics analyses of the interaction of polyclonal human and bat IgG with recombinant hFcRn and mFcRn. (A) Real-time interaction profiles depicting the binding of IgG from human or three bat species (*M. cappacinii, M. myotis*, *and N. noctula*) with immobilized hFcRn (left panels) and mFcRn (right panels). The depicted profiles were obtained after injection of IgG antibodies at concentrations of 0.195 – 25 nM. The black lines show the experimental data, the red lines represent the kinetic model fit obtained after global analyses of the experimental data using Langmuir binding model. All interactions were performed at 22 °C. (B) Values of kinetic parameters obtained after global analyses of the real-time interaction curves depicted in A. Each shown kinetic value represents the average value obtained after three independent kinetics fits. (C) Effect of pH on binding of human and bat polyclonal IgG to hFcRn and mFcRn. Real-time interaction profiles obtained after injection of 50 nM of human and bat IgG antibodies over sensor chip with immobilize hFcRn and mFcRn. The interaction analyses were alternatively performed at pH 6.0 (red lines) and 7.4 (black lines). All measurements were performed at 22 °C.

Figure 2. Sequence alignment (A) and identity index (B) of protein sequence of mouse (*Mus musculus*, gene BAA07110.1), bat (*Myotis myotis*, gene XP_036163001.1) and human (*Homo sapiens*, gene NP_004098.1) FcRn receptors.

Figure 1



b.

	Human FcRn			Mouse FcRn		
	k_a mol^{-1} s^{-1}	k_d s^{-1}	K_D, nM	k_a mol^{-1} s^{-1}	k_d s^{-1}	K_D, nM
H. sapiens	6.9×10^5	2.8×10^{-3}	4	4.3×10^5	9.9×10^{-5}	0.2
M.capaccinii	1.6×10^5	3.0×10^{-3}	18.6	2.6×10^5	7.4×10^{-4}	2.8
M. myotis	1.1×10^5	1.3×10^{-3}	11.4	9.1×10^4	1.0×10^{-4}	1.1
N. noctula	3.1×10^5	3.2×10^{-3}	10.5	2.6×10^5	3.5×10^{-5}	1.3

Figure 2

a.

FcRn Protein Sequence Alignment

```
Mus musculus    --MGMPLPWALSLLLVLLPQTWGSETRPPLMYHLTAVSNPSTGLPSFWATGWLGPQQYLT   58
Myotis myotis   MRVPRPQPWGLCLLLVLLPRTLRADGHRSLLYRLTAVSSPAPGSPAFWATGWLGPQQYLR   60
Homo sapiens    MGVPRPQPWALGLLLFLLPGSLGAESHLSLLYHLTAVSSPAPGTPAFWVSGWLGPQQYLS   60
                  :  * **.* ***.*** :  :: :  *:*:*****.*: * *:**.:*********

Mus musculus    YNSLRQEADPCGAWMWENQVSWYWEKETTDLKSKEQLFLEALKTLEKILNGTYTLQGLLG   118
Myotis myotis   YSDLRGQAEPFGAWIWESQLPWYWEKETADLRVKQALFLEAFTVLEE--GGSYILQGLLG   118
Homo sapiens    YNSLRGEAEPCGAWVWENQVSWYWEKETTDLRIKEKLFLEAFKALGG--KGPYTLQGLLG   118
                *..** :*:* ***:**.*: *******:**: *: *****:..*     * * ******

Mus musculus    CELASDNSSVPTAVFALNGEEFMKFNPRIGNWTGEWPETEIVANLWMKQPDAARKESEFL   178
Myotis myotis   CEMGPDNSTSAVATFALNGEEFMKFDPKAGNWDGDWPEARAISQKWKQHEDAVWQEGHFL   178
Homo sapiens    CELGPDNTSVPTAKFALNGEEFMNFDLKQGTWGGDWPEALAISQRWQQQDKAANKELTFL   178
                **:. **::  .* *********:*: : *.* *:***:  ::: * :: .*. :*  **

Mus musculus    LNSCPERLLGHLERGRRNLEWKEPPSMRLKARPGNSGSSVLTCAAFSFYPPELKFRFLRN   238
Myotis myotis   LTSCPQRLLGHLETGRSNLEWKEPPSMRLKARPGGPGLSVLTCSAFSFYPPELQLRFLRN   238
Homo sapiens    LFSCPHRLREHLERGRGNLEWKEPPSMRLKARPSSPGFSVLTCSAFSFYPPELQLRFLRN   238
                * ***.**  *** ** ****************.. * *****:*********::*****

Mus musculus    GLASGSGNCSTGPNGDGSFHAWSLLEVKRGDEHHYQCQVEHEGLAQPLTVDLD-----SS   293
Myotis myotis   GLAVGAGDSNFGPNGDGSFHAWSTLAVRSGDEHHYRCVVQHAGLPQPLVVGLGEVPRDSS   298
Homo sapiens    GLAAGTGQGDFGPNSDGSFHASSSLTVKSGDEHHYCCIVQHAGLAQPLRVELE-----SP   293
                *** *:*: . ***.****** * * *: ****** * *:* ** *** * *      *

Mus musculus    ARSSVPVVGIVLGLLLVVVAIAGGVLLWGRMRSGLPAPWLSLSGDDSGDLLPGGNLPPEA   353
Myotis myotis   AKSMVPVVGIVIGFLLLMAVAAGGAVLWWRMKRGLPAPWILLRGDDLGALLPTGPSKDAD   358
Homo sapiens    AKSSVLVVGIVIGVLLLTAAAVGGALLWRRMRSGLPAPWISLRGDDTGVLLPTPGEAQDA   353
                *:* * *****:*.**: .. .**.:** **: ******: * *** * ***

                EPQGANAFPATS        365
                S-----------        359
                DLKDVNVIPATA        365
```

b.